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EFFECT OF MICROGRAVITY ON EPIDERMAL DEVELOPMENT IN THE RAT

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I. What were the specific aims of the original study ?

The overall goal of this project was to investigate the effects of prolonged weightlessness on the development of the skin in the fetal and newborn rat. Specifically, we used the NASA microgravitational rat model to test the following hypotheses:

- (1) Exposure of the pregnant rat to microgravity during late gestation will diminish the transport of calcium across the placenta from the mother to the fetus leading to decreases in total epidermal and dermal calcium content;
- (2) Microgravity will lead to slowing of body growth and diminish the rate of formation of the outermost layer of the epidermis, the stratum corneum; and,
- (3) Microgravity will lead to formation of a stratum corneum with decreased DC electrical resistance and increased permeability to tritiated water.

II. What problems were encountered during the performance of this study ?

Animal dissection and collection of skin samples for analysis were successfully completed at both the Kennedy Space Center and the Dryden Research Facility. No major problems were encountered in our ground operations and both the NASA and Lockheed personnel were efficient and provided a ready source of information for our inquiries. Frozen tissue samples and fixed specimens were packed and shipped from both facilities. All samples were received intact and in good condition. Following arrival of the samples, no significant problems were encountered during tissue processing or data analysis.

The only problem occurring during the NIH.R1 mission was secondary to the weather-related landing at Edwards Air Force Base. The recovery of flight animals at a separate site from our ground controls necessitated foregoing experiments to determine surface hydration using bioinstrumental measurement of skin surface electrical capacitance. These experiments are best performed using a single observer and a single instrument to avoid possible errors resulting from individual technique or

instrumental calibration. Future studies of epidermal barrier maturation should include this simple but highly informative non-invasive measurement of water binding to the skin surface.

III. What were the results of the study ?

Morphological studies of skin development in flight and control animals were completed using transmission and scanning electron microscopy. Careful morphological cross-comparisons of flight and control animals were performed. Comparisons of scanning and transmission electron micrographs of fetal and postnatal animals did not conclusively reveal any uniform differences between the age-matched experimental groups. Light photomicroscopy was also utilized to evaluate the development of the epidermal barrier (J Invest Dermatol 100:400-406, 1993). The focus of these experiments was to test the hypothesis that conditions of space flight and microgravity would alter the number of layers constituting the outer structural barrier of the skin; i.e. the stratum corneum.

The results of these experiments are listed in Table 1. Data are reported as mean number of layers +/- standard error. Group means were analyzed by one-way analysis of variance and pair-wise comparisons were made using the Student-Newman-Keuls method. On average, the flight group pups possessed a significantly greater number of stratum corneum layers than any of the ground-based control groups ($p < 0.05$).

Table 1. Number of layers in the dorsal stratum corneum of newborn rat pups. (Flight samples taken on the day of birth following 3 days re-adaptation to terrestrial gravity).

	Nominal flight	Synchronous controls	Vivarium controls w/o hysterectomy	Vivarium controls w/ hysterectomy
mean $\pm$ sem	13.1 $\pm$ 0.4 *	11.7 $\pm$ 0.3	11.1 $\pm$ 0.2	12.0 $\pm$ 0.4
N	9	10	12	11

Calcium assays by atomic absorption spectrophotometry were completed and all samples read within the expected range. (J Appl. Physiol. 73(2):458-464, 1992).

Results from fetal and postnatal specimens are shown in Tables 2 & 3, respectively. The data are reported as means $\pm$ standard error in the units, mg calcium per 100 grams tissue wet weight.

As shown in the Table 2, fetal whole skin samples showed a statistically significant increase in calcium content compared to whole skin samples taken from the two sets of ground-based controls. This result was significant at the $p < 0.05$ level using ANOVA. It should be emphasized the results in Table 2 are for *whole* skin samples (epidermis plus dermis). The epidermis in fetal rat pups on gestational day 20 is very thin and fragile with little to no stratum corneum. Unlike term animals, the epidermis cannot be easily separated and studied in isolation from the dermis.

Table 2. Total calcium content of whole skin samples (epidermis + dermis) of fetal rat pups. Flight samples obtained immediately following shuttle touchdown. Results are mean $\pm$ SEM and the number of animals is shown in parentheses.

	Nominal flight	Synchronous controls	Vivarium controls
Whole skin	$7.70 \pm 0.16^*$ (15)	6.51 ± 0.15 (16)	7.03 ± 0.16 (19)

In marked contrast, as shown in Table 3 below, the epidermis of the flight animals was decreased in calcium content compared to the ground-based controls ($p < 0.05$, ANOVA). Calcium levels in the dermis and whole skin showed no statistically significant difference between groups. The epidermis in these experiments was isolated from the underlying dermis by heat separation at the time of dissection.

Table 3. Total calcium levels in the dorsal epidermis, dermis, and whole skin of flight vs control newborn rat pups. All animals were sacrificed shortly following natural birth. Results are mean + SEM and the number of animals is shown in parentheses.

	Nominal flight	Synchronous controls	Vivarium controls w/ hysterectomy
Epidermis	10.67 ± 0.36 * (12)	14.57 ± 0.79 (11)	14.34 ± 0.85 (11)
Dermis	6.27 ± 0.64 (14)	6.12 ± 0.15 (10)	6.35 ± 0.18 (14)
Whole skin	8.12 ± 0.24 (13)	8.64 ± 0.25 (13)	9.25 ± 0.37 (13)

Lastly, transport studies of water across whole skin and determination of skin electrical properties were measured on cryopreserved sections. Current-voltage profiles for skin excised from newborn rat pups were generated between -10 microamps to +10 micro amps. The effective electrical resistance of the tissues was calculated using Ohm's law. All samples tested showed high electrical resistance in the kilo-Ohm range consistent with values observed using fresh newborn rat skin samples in our laboratory in preliminary experiments. (Ph.D. thesis, Padmaja Shivanand, College of Pharmacy, University of Cincinnati, 1995). These results indicate good preservation of the tissue following transport from the dissection sites. Tritiated water flux across neonatal rat skin was measured under conditions of passive flux and during exposure to an iontophoretic field of 100 $\mu\text{A}/\text{cm}^2$ (active flux). Following a four-hour period of active flux, water transport was again measured under conditions of passive transport.

Overall, wide variations were noted within all groups and no major differences were observed with respect to the initial passive or active flux of water across the skin or in the baseline electrical resistance. Examination of the residual electrical resistance following application of an iontophoretic electrical field showed that the values were lower in skin membranes taken from the flight animals compared to all

other groups ($p < 0.05$, ANOVA). This result is consistent with a better barrier to water transport in the flight animals with less damage following application of an applied electrical field.

To summarize, the significant results of this project were:

1. Pregnancy in the Sprague-Dawley rat can be maintained under the adverse conditions of spaceflight and readaptation to terrestrial gravity;
2. No evidence of increased fetal wastage or somatic growth retardation was observed;
3. Vaginal delivery can be achieved following short term (3 days) readaptation to terrestrial conditions;
4. Fetal skin calcium levels are increased following development under conditions of microgravity;
5. Neonatal epidermal calcium levels are decreased following short term readaptation to terrestrial gravity;
6. Morphologically, the epidermal barrier in the late gestational rat exposed to microgravity and terrestrial readaptation is advanced by 12-24 hours;
7. Measurement of water flux and electrical resistance of the skin support the hypothesis of a better epidermal barrier in the flight animals compared to ground controls.

IV. Did these results differ from those predicted ?

The final experimental results differed markedly from those predicted. In earlier work we had demonstrated that alterations in uterine blood flow were associated with retardation in the rate of body growth in fetal rats (J Develop Physiology, 13:41-50, 1990). The overall study hypothesis, therefore, was predicated on the possibility of *unfavorable* alterations in uterine blood flow in the pregnant dams undergoing space flight coupled with the stress of take-off, adaptation to microgravity, and landing. We hypothesized that these stressors would result in a greater than normal rate of fetal

wastage and mild to moderate growth retardation of the surviving pups. Neither of these hypotheses was born-out by the resulting data. In contrast, animals appeared to do very well under the conditions of space flight. Pregnancy was sustained without untoward effect.

Based on our earlier findings, we also predicted that mild growth retardation in the flight animals would be accompanied by a diminution in the rate of epidermal barrier maturation. Quantification of the epidermal barrier would, hypothetically be manifested by a decrease in the numbers of layers in the outermost strata of the skin. Accompanying this decrease in the barrier component of the epidermis would be: (1) a decrease in calcium content of the skin and, (2) concomitant functional changes in barrier integrity marked by decreased electrical resistance and increased tritiated water flux. Again, our findings were diametrically opposed to the initial study hypothesis. In contrast, we found that the epidermis of the flight animals was accelerated compared to ground-controls as manifested by the significantly increased number of layers of the stratum corneum. This finding was corroborated by the observation that water transport and electrical resistance studies were indicative of a tendency for better barrier maturation in the flight animals.

The calcium data are particularly interesting. In contrast to our original hypothesis, skin calcium levels were actually increased in the flight animals compared to ground-controls. These skin samples were taken immediately upon landing of the shuttle and removal of one uterine horn from the mother in order to obtain the fetuses for analysis. Following re-adaptation to terrestrial gravity for three days, vaginal birth was allowed to occur normally (which it did). The epidermis was then removed from the dermis by brief heating, a procedure which is not possible in the fetal animals but is easily accomplished postnatally. Epidermal and dermal samples were then analyzed for relative calcium concentrations. Flight animals demonstrated a general decrease in calcium content in the epidermis. This is opposite to the direction of calcium content observed in fetal whole skin. Dermal sections from the postnatal animals showed no differences between groups.

V. How can the differences between the observed and predicted results be explained ?

In contrast to our original hypothesis, the conditions of space flight and re-adaptation to terrestrial gravity do not appear to be major stressors on the pregnant state. We can conclude that growth in the rats and development of the fetus can proceed normally under conditions of microgravity during the gestational period tested. It is noteworthy that the *available* room for habitation in the animal enclosure modules is increased under microgravity. It is possible that this factor may contribute to amelioration of the stress of overcrowding. In addition, *normal* vaginal delivery occurred following short term (three days) re-adaptation to terrestrial gravity. Whether such births would occur without the re-adaptation period is currently unknown.

In contrast to our original hypothesis, development of the outermost layer of the epidermis (the stratum corneum) was accelerated rather than retarded as originally hypothesized. This increase in the rate of maturation was indicated by the greater number of layers in the stratum corneum of the flight animals compared to ground-based controls. Functional parameters, such as water transport and electrical resistance, are also suggestive of a better epidermal barrier. The calcium data is of particular interest in so far as the calcium levels in fetal whole skin were significantly increased compared to ground-based controls. Calcium transport across the placenta in late gestational mammals is a facilitated process. It is likely, that mobilization of maternal calcium from bone occurred during space flight. It is possible, therefore, that the facilitated transport across the placenta was augmented by the presence of a surfeit of calcium derived from demineralized bone under conditions of microgravity. In contrast, re-adaptation to terrestrial gravity may have resulted in a reversal of this process such that epidermal calcium content was low compared to ground-based controls.

VI. What critical scientific questions have been answered or raised by this experiment?

The most important question answered by this experiment is the finding that mammalian pregnancy can be sustained under the stressful conditions of space flight and zero gravity. The incidence of fetal wastage was not increased in the flight animals nor was there evidence of somatic growth retardation. Short term adaptation to terrestrial conditions resulted in normal vaginal deliveries. These are important general findings.

The results of our specific study support the hypothesis that maternal gravitational exposure may effect organ specific differentiation in the fetus. Our findings of increased epidermal maturation may be the result of alteration in transplacental calcium homeostasis. These data support the hypothesis, therefore, of effects of microgravity, on non-bone calcium-dependent physiological processes. It has long been known that the skin is important in the control of systemic calcium metabolism and bone development. In rodents and humans, for example, a unique photoendocrine system localized to the epidermis regulates the cutaneous synthesis of vitamin D₃, the precursor of the important calcium regulatory hormone 1, 25, dihydroxyvitamin D₃. Calcium is also a critical regulator of cell division, cell adhesion, and programmed cell death within the epidermis. Calcium modulated genes are important regulators of the synthesis of the keratins, the major structural proteins of the epidermis.

In addition to its photoendocrine role, the skin may also function as a mechanoreceptive envelope (or body glove) with neurally-mediated links to the musculoskeletal system important for position and gravity sensing. In this novel view, the skin constantly transduces sensory information on the condition of the organism-environmental interface (friction, pressure, heat). This information is, in turn, translated by the central nervous system into patterns of motor output which then elicit new sensory inputs, etc. This view of the skin places the organism-environmental interface in the position of a critical *mediator* linking sensory signals to appropriate neuromuscular outputs. (Biosensors and Bioelectronics, 5:351-366, 1990)

VII. What are the implications of the results in terms of science and practical applications ?

Clearly, a major result of this experiment is the implication that mammalian pregnancy in humans can be sustained under conditions of space flight and zero gravity. These preliminary results lay the foundation for future NASA endeavors in which human pregnancy may be an important component, such as space colonization.

Our specific results in this project focus attention on the maturation of the skin and the possible role of calcium-dependent cellular processes to control the differentiation of this important physiological boundary. The results suggest that calcium transport across the placenta from the mother may be increased under microgravity, such that increased levels of calcium are present in the skin of the fetal animals. To our knowledge, there have been no investigations of basic epidermal biology and barrier maturation under conditions of space flight in humans. The skin is clearly a vital organ for temperature control, protection from ultraviolet light, prevention of water loss, and tactile discrimination. It may be warranted, therefore, to look further at the effects of space flight and zero gravity on the development of this important and accessible biological tissue.

VIII. Should NASA continue to pursue research in this area ?

As was noted in the original proposal, the epidermis forms the ultimate bioevolutionary "spacesuit" interfacing the human organism with his physical environment. Non-invasive instruments are currently available for quantifying physical properties of the outermost layer of the skin (the stratum corneum). Such instrumentation includes devices for measuring surface acidity, water content, hydrophobicity, visco-elasticity, frictional coefficient, desquamation indices and other important surface properties. The development of better non-invasive instrumentation for assessing skin surface physical properties may be a legitimate area to pursue in order to reach NASA objectives. For example, the assessment of physiological state during and following extra-vehicular activity (EVA) may be enhanced by skin-based

monitoring devices (temperature, blood flow, transepidermal water). Such NASA-based sensing systems may find parallel application in biomedical settings (for example, physiological monitoring in intensive care units). Studies in humans focusing on the epidermal-environmental interface are: (1) feasible, given the easy accessibility of the epidermis, (2) developmentally relevant, given this tissue's biological property of continual cellular replacement, and (3) practical, given the important role of the stratum corneum as a platform for non-invasive physiological monitoring.

IX. What benefits does NASA derive from developing expertise in the area of skin-based sensing and epidermal biology ?

Significant advantages may accompany the promotion by NASA of skin research. The concept that the skin forms the natural "spacesuit" for the body is an easy one for the public to grasp. From a biological standpoint, the skin is a complex and dynamic organ which is highly adaptive to changes in environmental conditions. The presence of a self-cleaning, self-replenishing boundary layer with sensing capabilities fits neatly into the field of autopoiesis; i.e. the theory of self-organizing systems. The strategic location of the skin between the body and the external environment (outerspace) makes it a logical target for information retrieval technologies. The development of new sensing systems using skin-based techniques should have practical spin-offs to the medical care environment as well as the skin care industry. Demonstrable reapplication of NASA-developed technologies would be expected to have a positive impact on future agency funding.